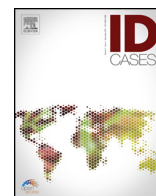


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Case report

Prolonged shedding of Chikungunya virus in semen and urine: A new perspective for diagnosis and implications for transmission



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ABSTRACT

We report the presence of Chikungunya (CHIKV) RNA in the blood, urine and semen during the acute phase of the disease in an adult with a dual infection with Dengue virus type 3. The patient, a 25 yr-old man from Salvador, Brazil, reported a 6-day duration of high fever, arthralgia, myalgia, headache and photophobia.

Blood and semen specimens were positive for CHIKV in the first collected samples; semen and urine specimens were positive for CHIKV after 30 days of symptoms onset. DENV-3 RNA was positive in blood specimen when first collected 6 days after the initiation of symptoms. We describe for the first time the presence of CHIKV RNA in urine and semen for an extended period of time and we address the possible implications of these findings for diagnosis and transmission dynamics.

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Introduction

Zika and Chikungunya viruses are both transmitted by *Aedes aegypti* mosquitoes and are associated with an abrupt onset of fever, arthralgia, myalgia and rash [1]. In Brazil, acute exanthematous outbreaks related to Zika (ZIKV) and Chikungunya viruses (CHIKV) have been reported in many regions and sometimes as co-infections [2–5].

Acute Chikungunya infection generally leads to prominent joint symptoms with moderate to severe arthralgia and arthritis that

may persist for months and even years [6]. Rarely, encephalitis and other neurological involvements may also complicate CHIKV infection such as recently reported a neonatal encephalitis in Brazil with similar lesions on brain scan as those previously described in the La Réunion Islands [7–9]. In all these situations, a rapid diagnosis is of importance for epidemiological purposes and clinical management.

In ZIKV infections, the molecular diagnosis in serum is generally possible until the first six days of symptoms onset and, later on, urine or saliva is used [10,11]. Zika detection in urine may help in ascertaining the diagnosis after the initial period of viremia in areas with co-circulation of ZIKV and Dengue virus (DENV), where serologic cross-reactions may occur, but evidence for that associated with CHIKV infection has not been shown [12]. An expanded window for diagnosis in CHIKV is also needed since IgM seropositivity may persist for months after the initial acute infection. Many other viruses have been detected in semen, such as Ebola, HIV, HBV, HPV, HSV-1,

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HSV-2, Marburg, but there has been no published data so far on the presence of CHIKV in semen [13–17].

We report the presence of CHIKV RNA for a prolonged period of time in the urine and semen of an adult with a dual infection with Dengue virus type 3 for a prolonged period of time.

Case

A 25-year-old man from Salvador, Brazil presented with a 6 days of high fever, arthralgia, myalgia, headache and photophobia. He also reported a burning sensation on his eyes and a non-pruritic rash in the trunk and upper limbs on the first 3 days, and complained of pain involving the ankles, knees, shoulders, wrists, lower back, and neck. Of note, he also reported a transient burning sensation in the urethra and genital region that started 3 days before the onset of symptoms and resolved spontaneously. On examination the patient was febrile with a temperature of 38.3°C, had a maculopapular erythematous diffuse rash, joint enlargement with mild inflammatory signs affecting the wrists, the metacarpophalangeal and metatarsophalangeal joints as well as the ankles. The patient was not able to raise his arms on command because of shoulder pain and was eating with difficulty because of pain involving his temporomandibular joint.

Blood samples were drawn for viral workup, semen was also requested due to the complaint of dysuria, and the possibility for Zika diagnosis since the patient could not provide a urine sample at that time. Blood, urine and semen were collected again after 30 days of symptoms onset and were further processed. Serum and semen samples were submitted to viral RNA extraction using a Maxwell Total Viral Nucleic Acid 16 Purification Kit (Promega, USA) regarding that the semen sample, the incubation time of the lysis step was extended to 30 min. Urine sample was submitted to viral RNA extraction using QiAmp viral RNA purification (Qiagen, USA). Subsequent to viral RNA extraction, all samples were submitted to RT-PCR technique (AccessQuick™ System, Promega, USA) to screen for CHIKV, ZIKV and DENV using conventional techniques [18–20].

Serum and semen specimens were positive for CHIKV RNA in the first collected samples; semen and urine specimens were positive for CHIKV RNA after 30 days of symptoms onset, with negative results in serum. DENV-3 RNA was positive in serum specimen when first collected 6 days after the initiation of symptoms and was not tested thereafter. All specimens tested negative for ZIKV.

Table 1 presents the clinical findings and molecular results during the time frame of infection. The patient made use of prednisone starting on the 2nd day of symptoms onset with 40 mg

QD for 10 days, tapering to 20 mg QD for the next 10 days, and 10 mg QD for the last 8 days, with an uneventful course and no clinical findings after 30 days.

Discussion

Many different viruses can be isolated in semen specimens for prolonged periods of time. Ebola RNA (EBO-RNA) can be detected in up to 290 days after clinical manifestations and Ebola infectious particles in up to 70 days [21]. Transmission of Ebola virus (EBOV) from a male survivor to a female has been documented 179 days after illness in Liberia [21]. In another study, persistence of EBO-RNA could be found in semen specimens at least 9 months after the onset of symptoms with important consequences related to sexual transmission [16]. Many papers have reinforced the sexual transmission of ZIKV since Foy et al. have suggested its relationship [22–25]. This report, besides describing a patient with classical signs of ZIKV, drew attention to symptoms of prostatitis with hematospermia [25]. Our patient reported a burning sensation in the urethra and the genital region and may also have had a transient prostatic involvement with detection of CHIKV RNA in semen. ZIKA RNA has been detected in semen for up to 6 months after the onset of symptoms and is involved in male-to-female as well as in female-to-male transmission [26,27].

The precise replication site for many of the viruses found in semen is unknown. Luttmer et al. studying Human Papilloma virus (HPV) have found a high prevalence of this virus in semen – 27.2% of healthy adults in Amsterdam – and using fluorescence in situ hybridization (FISH) for HPV-DNA and immunocytochemistry for the HPV-L1 and HPV-E4 proteins, the authors detected HPV-DNA, HPV-E4 and HPV-L1 in exfoliated keratinocytes present in some HPV16-positive semen samples, indicating the presence of HPV viral particles [28].

Even though *Alphavirus* envelope glycoproteins function as the attachment point to cells, no human cell surface receptor has been implicated in cell entry so far.

Primary peripheral blood cells such as CD4+ T lymphocytes, primary CD14+ monocytes and dendritic cells were reported to be refractory to CHIKV binding and infection [29]. Macrophages, on the other hand, are highly sensitive to CHIKV and showed cytopathic effect following CHIKV infection [29,30]. Mononuclear cell infiltration and viral replication in the muscles (particularly skeletal muscle progenitor cells, not muscle fibers) and joints are associated with myalgia and arthralgia and synovial macrophages have been shown to contain viral RNA months after infection [31]. It may be postulated that an inflammatory infiltration with macrophages in the genital tract may be the source of the CHIKV,

Table 1
Molecular markers and clinical course during acute dual infection with DENV-3 and CHIKV.

Time from symptoms onset (days) ^a	Symptoms	DENV (serum)	CHIKV (serum)	CHIKV (urine)	CHIKV (semen)	ZIKV (serum)	ZIKV (urine)	ZIKV (semen)
–3	Urethral burning	nd ^b	nd	nd	nd	nd	nd	nd
0–+6	Fever	Pos+	Pos+	nd	Pos+	Neg–	Neg–	Neg–
	Arthralgia							
	Headache							
	Burning eyes							
	Rash							
+30	Resolved	nd	Neg–	Pos+	Pos+	Neg–	Neg–	Neg–

Pos+ means a positive result.

Neg– means a negative result.

^a A negative sign preceding a numeral meaning the number of days before initiation of CHIKV/DENV symptoms.

^b not done.

leading to mild symptoms and an unknown persistence time in semen. The detection of CHIKV in semen has many important implications, including the possibility of sexual transmission. Even though we did not perform cytopathic assays for CHIKV, this finding must be further investigated. We did not find any other study showing evidence of CHIKV in semen and, specially, for such a long period since the onset of symptoms.

Another unique finding in our study was the detection of CHIKV in urine after 30 days of the initial symptoms contrasting with recent findings in literature showing no detection of CHIKV in urine after the first week of symptoms onset [32]. This could provide an expanded window for the diagnosis of a recent infection with CHIKV. The use of prednisolone may have contributed to the prolonged viral shedding and another factor that may, in part, have added to that was the possible interaction between Chikungunya and Dengue type 3. With the ongoing circulation of DENV, CHIKV and ZIKV in Brazil, such a finding of dual infections will be increasingly seen and reported, as we recently published for CHIKV and ZIKV [5]. In India, an estimate of co-infection with CHIKV and DENV using serological markers estimated point prevalences on the range of 5.7–9.5% [33]. In 1964 Myers and Carey reported that seven patients out of 332 patients (2.1%) from south India had a simultaneous increase in antibody to both CHIKV and DENV virus and that these patients had also an unremarkable clinical course and an expected development of antibodies to both agents, indicating that the host response was not altered by the dual infection [34]. During an outbreak of both CHIKV and DENV-1 in Toamasina, Madagascar, in 2006, 10 out of 55 patients sampled (18.2%) were shown to be co-infected with both virus with no complications recorded [35]. However, Chahar et al. reported that during the 2006 dengue outbreak in Delhi six patients were co-infected with CHIKV and DENV (with DENV-3 in 5 out of 6), 2 of these patients had dengue hemorrhagic fever with central nervous system (CNS) involvement and one patient died [36]. More recently, Villamil-Gomez et al. reported a 49-year-old male from Colombia with a febrile illness, bilateral conjunctivitis and a pruritic rash that was diagnosed with ZIKV, CHIKV and DENV and had an uneventful course [37]. The true extent of CHIKV-DENV co-infection has been hampered by current diagnosis largely based on clinical grounds with unavailability of molecular methods in many parts of the world [38,39].

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Consent section

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Conflict of interests

All authors: No reported conflicts.

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